host lymphocytes and consistent with our recent observations in short-term cultures (Luznik, L. et al: Blood; in press). Interestingly, AH1-specific allogeneic and host CD8+ T cells, expanded from the mixed chimeras had a higher affinity than the T cells derived from the vaccinated nontransplanted mice. In conclusion, mixed chimerism provides an optimal platform for immunotherapy of solid tumors not only because of allogeneic effect but also through augmentation of host tumor-specific immunity.

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Vβ REPERTOIRE ANALYSIS OF TISSUE INFILTRATING CD8+ TCELLS RESPONDING TO MINOR HISTOCOMPATIBILITY ANTIGENS INVOLVED IN GRAFT-VERSUS-HOST DISEASE

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Lethal graft-versus-host disease (GVHD) can be induced between MHC-matched murine strains expressing multiple minor histocompatibility antigen (miHA) differences. In the B6-> BALB.B model, both CD4+ and CD8+ donor can mediate lethal GVHD, whereas in the B6->CXBE model (expressing a subset of the BALB.B miHA), only the CD8+ T cells are lethal. CDR3-size spectratyping was used to analyze the CD8+ and CD4+ T cell responses in both BALB.B and CXBE recipients injected with B6 CD8+ or CD4+ T cells. Spectratype analysis of the reacting CD8+ T cells indicated overlapping skewing of Vβ1, 4, 6, 8-10 and 14 families in both the CXBE and BALB.B recipients and unique skewing of the Vβ 4 family in the BALB.B recipients. The reacting CD4+ T cells exhibited overlapping expansion of Vβ4, 6-10, and 12-14 families, but the B6->BALB.B response also appeared to recognize unique BALB.B-specific miHA, indicated by additional skewing of Vβ2 and 11. Positively-selected TCR Vb skewed CD4+ and CD8+ T cell subsets injected into lethally irradiated BALB.B recipients were capable of inducing fatal GVHD. BALB.B mice transplanted with non-skewed Vβ CD4+ T cells survived with minimal symptoms of GVHD. Here we examine the T cell repertoire responses involved in target tissue damage. Infiltrating B6 host-presensitized CD8+T cells were isolated post-transplant from the intestines, livers and spleens of lethally irradiated CXBE and BALB.B recipients. The results indicated overlapping tissue skewing between the B6->BALB.B and B6->CXBE combinations for Vβ3, 5, 6, 14, and 18 in the spleens, for Vβ5, 9 and 13 in the intestine and Vβ2 and 18 in the liver. To test the possibility that reducing T cell responses within a critical GVHD target tissue might diminish GVHD lethality we used magnetic sorting to remove the intestinal infiltrate skewed Vβ families from the transplant inoculum. Mice transplanted with non-skewed Vβ CD8+ T cells exhibited a two-fold increase in median survival time. Taken together these results suggest that differential targeting of T cell responses can be exploited to help avoid GVHD.

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DENDRITIC CELLS CULTURED WITH INTERFERON PLUS GM-CSF CAN BE USED TO ELICIT PR1-SPECIFIC CTL FOR ADOPTIVE IMMUNOTHERAPY

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We sought a more efficient method to produce potent dendritic cells (DCs) that could be used to elicit PR1-CTL for adoptive immunotherapy. Peripheral blood mononuclear cells (PBMC) from healthy HLA-A2+ donors were obtained by Ficoll-Hypaque separation and monocytes were obtained after two-hour plastic adherence. Monocytes were treated for seven days in RPMI medium plus 10 % human AB serum (HS) with interferon-alpha2b (IFN-a) at 1000 units/ml and GM-CSF at 500 units/ml (IF/GM-DC). On day seven, the cultures showed a mature phenotype similar to DC grown in the same culture media with IL-4 (1000 units/ml) and GM-CSF (500 units/ml) (I4/GM-DC): CD83 (41.9% vs. 39.4%), CD11c (90.8% vs.85.23%), CD86, (26.5% vs. 53.88%) and CD80 (21.9% vs. 35.49%). To compare PR1-specific CTL elicited using either DC population, IF/GM-DC and IL4/GM-DC were pulsed with PR1 at 20 mg/ml, radiated, and incubated at a 1:2 ratio with autologous PBMC from HLA-A2+ healthy donors. On day 7, 14 and 21 the co-cultures were restimulated with PR1-pulsed DC, and IL-2 (20 IU/ml) was added the following day. PR1-CTL cultures from 6 donors stained with PR1/HLA-A2 tetramer showed 0.16% to 5.83% (mean = 1.83%) tetramer+ CD8+ lymphocytes from the IF/GM-DC cultures vs. 0.04% to 7.4% (mean = 1.92%) from the I4/GM-DC cultures ($p>0.05$). IF/GM-DC induced potent PR1-CTL proliferation with a stimulation index of 2.17 compared with 0.19 for lymphocytes alone using BrdU incorporation. Mean cytotoxicity results from five separate donors showed peptide-specific lysis of 33.8% for PR1-coated T2 cells compared with 6.5% for pp65-coated T2, at an effector:target ratio of 20:1 by micro-cytotoxicity assay. These results show that IFN-a and GM-CSF can be used to elicit mature DC cells from PBMC that can be used to elicit PR1-CTL after only 21 days of culture.

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PHASE III TRIAL WITH INFLIXIMAB/METHYLPREDNISOLONE (MP) VS MP FOR THE TREATMENT OF ACUTE GVHD: PRELIMINARY FINDINGS

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Preclinical and human studies of acute GVHD (aGVHD) suggest that tumor necrosis factor-alpha (TNF-a) may play an important pathogenic role in aGVHD. We have previously reported a high response rate in the treatment of steroid-refractory aGVHD with infliximab, a chimeric human-mouse anti- TNF-a monoclonal antibody. In this study, we evaluate the efficacy and toxicity of infliximab in the upfront treatment of aGVHD. Methods: Single institution, open-label, randomized, controlled trial that compares the combination of infliximab with MP versus MP in the upfront treatment of grade 2 or higher aGVHD. After stratification according to donor type and the presence or absence of GI involvement, patients were randomized to receive MP (2 mg/kg/day with standardized taper) plus infliximab (10 mg/kg weekly x 4 weeks) or MP alone. Results: The trial has currently enrolled 52 patients with an accrual goal of 100 patients. Of 39 patients evaluable for response, the overall response rate of GVHD was 63% (12/19) in the MP + infliximab arm and 50% (10/20) in the MP arm. CR was observed in 11/12 treated with MP+ infliximab, and all 10 responders in those receiving MP alone (p=NS). In the infliximab group responses were observed in aGVHD of the skin (n= 10) and the GI tract (n= 5). Fourteen (60%) patients in the MP+ infliximab arm developed one or more bacterial, viral or fungal infections compared to 12 (60%) in the MP arm (p= NS). Median survival was 208 and 168 days for the MP+ infliximab and MP groups respectively, and no statistical differences in mortality have been observed between the two arms. Number and causes of deaths are shown in the Table. Conclusions: So far, both the MP+ infliximab and MP arms show a similar profile of efficacy, toxicity and relapse rate. Both arms have a comparable and relatively high prevalence of infection, highlighting the need for aggressive monitoring and prophylaxis in this population. This study continues accrual.

	Death%(n)	Cause:(n) GVHD	Infection	Fungal inf.	Relapse
MP/Inf.	60% (14/23)	8	4	1	2
MP	75% (15/21)	8	2	1	6

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ENGRAFTMENT, GVHD, IMMUNE RECONSTITUTION AND SURVIVAL FOLLOWING CD8+ T CELL DEPLETED ALLOGENEIC PERIPHERAL BLOOD STEM CELL TRANSPLANTATION (PBSCT)

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Partial depletion of CD8+ T cells from donor lymphocyte infusions reduces the incidence of GVHD without compromising